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ORIGINAL ARTICLE

An osteological assessment of cyclopia by micro-CT scanning

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Abstract

Purpose Imaging modalities such as micro-CT scanning and three-dimensional reconstruction are providing a mechanism for detailed analysis of skeletal components not only of normal specimens but also through revisitation of the abnormal. The aim of this study was to analyse the craniofacial skeleton of five human fetuses with cyclopia by means of micro-CT scanning and three-dimensional reconstruction.

Materials and methods The study consisted of five cyclopean individuals from the paediatric collection of the School of Anatomical Sciences, University of the Witwatersrand. The specimens ranged in age from 22 to 42 weeks of gestation. The osteological features of each bone of the skull were analysed with the aid of micro-CT scanning and analysis using VG studiomax software.

Results A detailed analysis of all the bones of the skull revealed that the upper two-thirds of the viscerocranium and the anterior region of the basicranium were the most affected regions of the cyclopean fetuses. The ethmoid, nasal, inferior concha and the lacrimal bones were absent in all the cases of cyclopia. Major abnormalities were found in the premaxillary region which affected the development of the anterior dentition.

Conclusion This study supports the suggestion that the malformations of the visceral bones are secondary to defective development of the presphenoid and mesethmoid cartilages. The ethmoidal bones are important midline struts during normal development and their absence in cyclopia leads to non-laterality of facial features.

Keywords Cyclopia · Osteology · Craniofacial development

Introduction

Developmental disturbances often highlight pathological conditions [1] while providing clues to normal development. Such a condition is cyclopia which allows for the study of complex craniofacial development and morphology through its very disturbance of the elements of the region.

Cyclopia is said to be the most acute form of holoprosencephaly [7] with a prevalence of 1 in 16,000 live births [1]. Fetuses presenting with this abnormality have a single median orbit, arhinia, and a proboscis (a tubular appendage, representing nasal tissue), generally situated superior to the median orbit [12, 19, 20]. The absence or incomplete "cleavage" (actually division into two telencephalic vesicles) of the prosencephalon which results in holoprosencephaly occurs as early as the 18th–28th day of gestation [27], but the aetiology of the condition remains unclear. While maternal diabetes mellitus has been implicated [8], other factors have also been associated [8], but have not been shown to be causative [16]. Genes, for example, SHH [6], are known to be involved in the condition. As holoprosencephaly is a midline defect resulting from the inability of the forebrain to divide into two cerebral hemispheres [8], it affects other midline structures including the craniofacial skeleton during the course of its development.

Many of the midline skeletal structures are compromised in cyclopia and result in variations of the bony composition of the floor of the median orbit. McGrath and Sperber [18] through multi-planar sectioning of cyclopean fetuses observed that the floor of the single median orbit in the anterior median plane was composed of the superior surface of the maxillae, the lacrimals and the orbital processes of the palatine bones, while the lateral thirds of the orbital floor were formed by the maxillae and zygomatic bones [18].

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Kokich et al. [13] by sectioning and radiography observed the vomer bone as a thick mass which merged with the paired maxillary bones to form the floor of the median orbit, while Situ et al. [25] utilizing magnetic resonance imaging of a single cyclopean fetus observed the inferior concha (turbinate) and maxilla to have developed normally, but with a rudimentary vomer positioned superior to the maxilla. While variations in the craniofacial development of fetuses with cyclopia have been described, it is unclear if these are the result of individual variation or the methods available to researchers at the time.

Advances in imaging technology have provided an avenue to further investigate the complexities of disparities associated with cyclopia. Some investigations of cyclopia have included magnetic resonance imaging [15, 25] and while providing details of the soft tissue relationships associated with cyclopia, the technology fails to highlight the variations in the osteology of the cyclopean skeleton. In contrast computed tomography, i.e. cone beam CT, micro-CT has proven to be a useful tool for investigating both the external and internal variations arising from the development and growth of the skeleton [22] and cyclopia in particular [15]. As most cyclopean fetuses die prenatally or live for an average of 4 h after birth, ossification of the bones of the skull has not been completed [25]. Thus, micro-CT technology has provided researchers with the opportunity to study detailed osteology of cyclopia in three dimensions while preserving the integrity of the specimens.

To derive an understanding of the dysmorphology which occurs, and to gain a better understanding of the development and growth of the craniofacial complex, the components of the craniofacial skeleton of fetuses with cyclopia were analysed with the aid of a micro-CT scanner.

Materials and methods

The study made use of paediatric cadavers sourced from the School of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand. Ethical clearance to undertake the study was granted by the Human Research Ethics Committee (Medical) (W-CJ-140604-1).

The study included five prenatal cadavers (numbered fetus 1–5) depicting cyclopia. While the brain was not studied in these individuals, the definition used is that of Müller and O'Rahilly [19] and Cohen and Shiota [8] in which the condition of cyclopia is accompanied by holoprosencephaly. The individuals (three females and two males) ranged in age from 24 to 42 weeks of gestation. In addition, one normal individual of similar age presenting with no diagnosed craniofacial abnormalities was scanned for comparison with the cyclopean fetuses (Fig. 1). Age estimation of the fetuses was

Fig. 1 Anterior view of cyclopean fetuses, indicating sex and gestational age (gw) of each fetus. Note the single, median orbit and absence of a nasal cavity in each specimen

determined using standard measurements, e.g. crown–rump length, crown–heel length, toe–heel length and head circumference. No demographic data on any of the specimens or their mothers are on record in the school. Dissection could not be undertaken to ensure integrity of the specimens for future research endeavours.

Each fetus was scanned using a Nikon XTH 225L micro-focus CT X-ray unit (Nikon Metrology, Leuven, Belgium), located at the MIXRAD laboratory of the South African Nuclear Energy Corporation (Fig. 2), Pelindaba. A 0.1-mm-thick silver filter was used to approximate a homogenous X-ray beam spectrum, by eliminating the lower energy photons. Each fetus was mounted vertically and secured in place with polystyrene. The container was then placed on to a rotating sample manipulator within the micro-CT scanner which facilitated scanning at 360°. A thousand projection images were obtained for each fetus.

The scanning parameters, e.g. scan resolution and tube voltage, were set in accordance with the largest fetus as a means of standardizing the scanning conditions across the sample. The scans were then converted into a stack of cross-sectional image slices using Nikon CT Pro software to generate a 3-D image (Fig. 3). Subsequent to reconstruction, the scans were imported into VG Studio Max V2.2 (Volume Graphics GmbH, Heidelberg, Germany) as volume files for further examination. A second reconstruction was done to isolate the individual skull bones and to analyse the morphology of each bone.

In all the cases of cyclopia, an assessment of each bone in the viscerocranium and the neurocranium, and its respective morphological features was made. In addition, the orientation, location and fusion of the craniofacial bones were analysed.

Fig. 2 Interior view of the Nikon XTH 225L micro-focus CT X-ray unit beam emitter and rotation plate utilized in the scanning of the fetuses

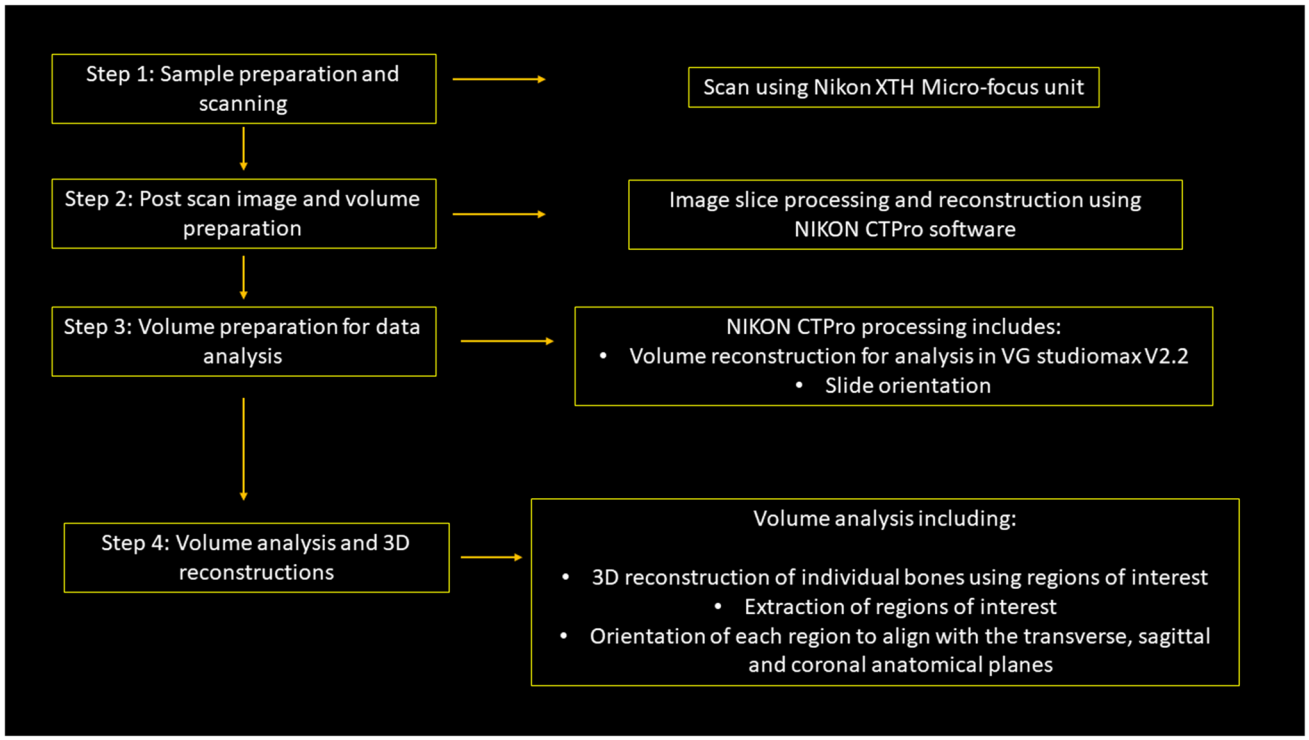

Fig. 3 Flow diagram indicating the process of scanning, 3D-reconstruction and volumetric analysis of specimens

Results

As seen with micro-CT scanning and three-dimensional reconstruction, the detailed osteology of each skeletal element associated with the cyclopean craniofacial complex is highlighted in Table 1. In all the fetuses with cyclopia in the present study, a single median orbit and the absence of a nasal cavity (Fig. 1) were noted. A proboscis was present above the single median eye in each fetus, but included no skeletal elements.

In general, the bones of the neurocranium did not appear to be affected with the exception of the frontal bone (Table 1), which was highly variable in its morphology. In particular, the frontal bone of fetus number 4 appeared to be at an earlier stage of osteogenesis, yet was the oldest

Table 1 Variations in the morphology of the neurocranial and viserocranial elements of each of the five cyclopic individuals

Neurocranium						
Bone	Feature	Fetus 1	Fetus 2	Fetus 3	Fetus 4	Fetus 5
Occipital	Pars Squama	N				
	Pars laterali	N				
	Pars Basilaris	N	ABN: Triangular in shape, fused to postsphenoid	N		
Temporal	Squamous	N		ABN: displaced laterally, reducing the size of the infra-temporal fossa	N	
	Tympanic	N				
	Petrous	N		ABN: Semi-circular canals distorted	N	
	Mastoid	N				
Parietal		N				
Frontal	Nasal process*	ABS				
	Zygomatic process*	ABN: Deficient				
	Metopic suture	ABN: partially fused	N	ABN: Fused	ABN: Partially fused	
	Orbital plate	Two separate plates		ABS	ABN: partial appearance of a right orbital plate with very thin bone	ABN: both orbital plates are partially formed
	Orbital margin	well defined supra-orbital margin thickened anteriorly	well defined supra-orbital margin	ABS		ill-defined supra-orbital margin
	Ethmoid notch	Present	ABS			Present
Viscerocranium: Orbit						
Zygomatic	Frontal process	N				
	Maxillary process	N				
	Temporal process	N		Sharpened	N	
	Zygomatic notch	N				
	Orbital surface	N		Reduced	N	
	Temporal surface	N				
Sphenoid	Pre-sphenoid	ABN: reduced jugum				
	Post-sphenoid	ABN: tuberculum and dorsum sellae present, defined sella turcica				ABN: tuberculum and dorsum sellae present, shallow hypophyseal fossa, well defined anterior clinoid processes, well defined internal carotid sulcus
	Lesser wings	ABN: underdeveloped with a central optic canal, jugum and wings appear to be fused in a central bony mass	ABN: underdeveloped with a central optic canal	ABN: no central optic canal, jugum and lesser wings appear as one bony mass	ABN: long vertically oriented lesser wings, no central optic canal but smaller incomplete optic canals infero-lateral to the lesser wings	ABN: underdeveloped with a central optic canal, small degree of separation between wings just superior to the central optic canal
	Greater wings	ABN: Malformed superior orbital fissure		ABN: No evidence of foramen rotundum, ovale or spinosum, no evidence of pterygoid plates, Malformed superior orbital fissure, intracranial concavity of orbital surface is shallow	ABN: Bone on the orbital surface is very thin, Malformed superior orbital fissure, intracranial concavity of orbital surface is shallow, greater wings appear to be inverted anteriorly	ABN: Bone on the orbital surface is very thin, Malformed superior orbital fissure, intracranial concavity of orbital surface is shallow,

Table 1 (continued)

Viscerocranium: Nasal Cavity				
Lacrimal		ABS		
Ethmoid		ABS		
Vomer		ABS		
Inferior nasal concha		ABS		
Nasal		ABS		
Viscerocranium: Oral Cavity				
Maxilla	Premaxilla	ABN		
	Dental crypts	One conoid shaped central incisor, no lateral incisors, both canine teeth as well as a full set of deciduous molars present	Central incisors present, lateral incisors and canine crowns absent, full set of deciduous molars at different stages of development present	
	Anterior nasal spine	ABN		
	Frontal process	ABN		
	Zygomatic process	N	ABN	N
	Palatine process	Projected medially, markedly concave on oral surface		Projected medially, markedly concave on oral surface, very thin posteriorly
	Orbital surface	Horizontally aligned and fused in the midline		Horizontally aligned and fused in the midline, very thin posteriorly
Palatine	Perpendicular plate	Fused centrally together in midline forming a bony mass		
	Horizontal plate	Horizontally aligned	Horizontal plates present but under developed, greater and lesser palatine foramina present	
	Orbital process	Fused centrally to the bony fused bony mass of the perpendicular plate		
	Sphenoidal process	Fused centrally to the bony fused bony mass of the perpendicular plate		
	Pyramidal process	N		
Mandible	Body	Mental suture not fused, hemi-mandibles clear		Mental suture not fused, underdeveloped labial and buccal surfaces of hemi-mandible
	Ramus	N		Gonial eversion bilaterally, under developed mandibular notch
	Condylar process	N		Poorly developed, bone very thin
	Coronoid process	N		Poorly developed, bone very thin
	Dentition	Crowding of incisors, canine and both deciduous molars present		

N normal, *ABS* absent, *AbN* abnormal or underdeveloped

*Features present in adult but used for descriptive purposes and orientation

fetus in terms of gestational age in the group (Fig. 4). A normal patent metopic suture was only found in fetus 2, while a fused suture occurred in fetus 3 and variations of sutural closure appeared in the remaining fetuses (Table 1; Fig. 4). Further variations in the morphology of the frontal bone were predominantly the result of its association with the orbit. The floor of the central orbit was formed by the maxilla in all cases. The zygomatic bones and the greater wings of the sphenoid bone formed the anterolateral border and the posterolateral border of the orbit, respectively. The lesser wings and the partially developed orbital plates of the frontal bone formed the posterior and superior boundaries of the orbit, respectively.

A large degree of variation was observed across all components of the sphenoid bone (Table 1; Fig. 5). While the presphenoid bone (precursor to the body of the sphenoid bone) or parts thereof were present, the jugum was reduced in size in all cases. In addition, the morphology of the lesser wings was highly variable. No central optic canal was found in fetuses 3 and 4 (Table 1; Fig. 5). A tuberculum, dorsum sellae and sella turcica were present across all cases, with a shallow hypophyseal fossa occurring in fetus 5. In addition well-developed anterior clinoid processes and internal carotid sulci were observed in fetus 5 (Table 1).

As the ethmoid bone was not present across all the cyclopean fetuses, no evidence of a cribriform plate was found.

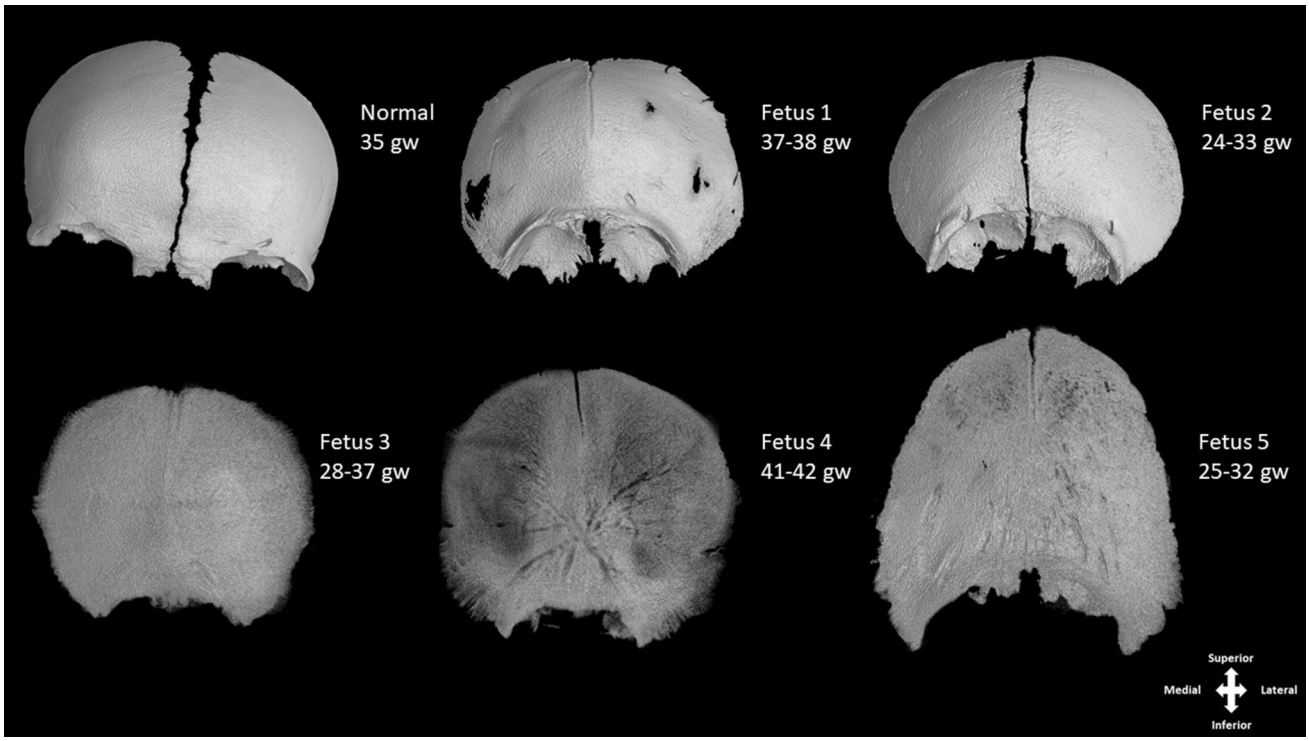

Fig. 4 Anterior view of the frontal bone of a normal and the five cyclopean fetuses, indicating the variability of this bone in the cyclopean fetuses

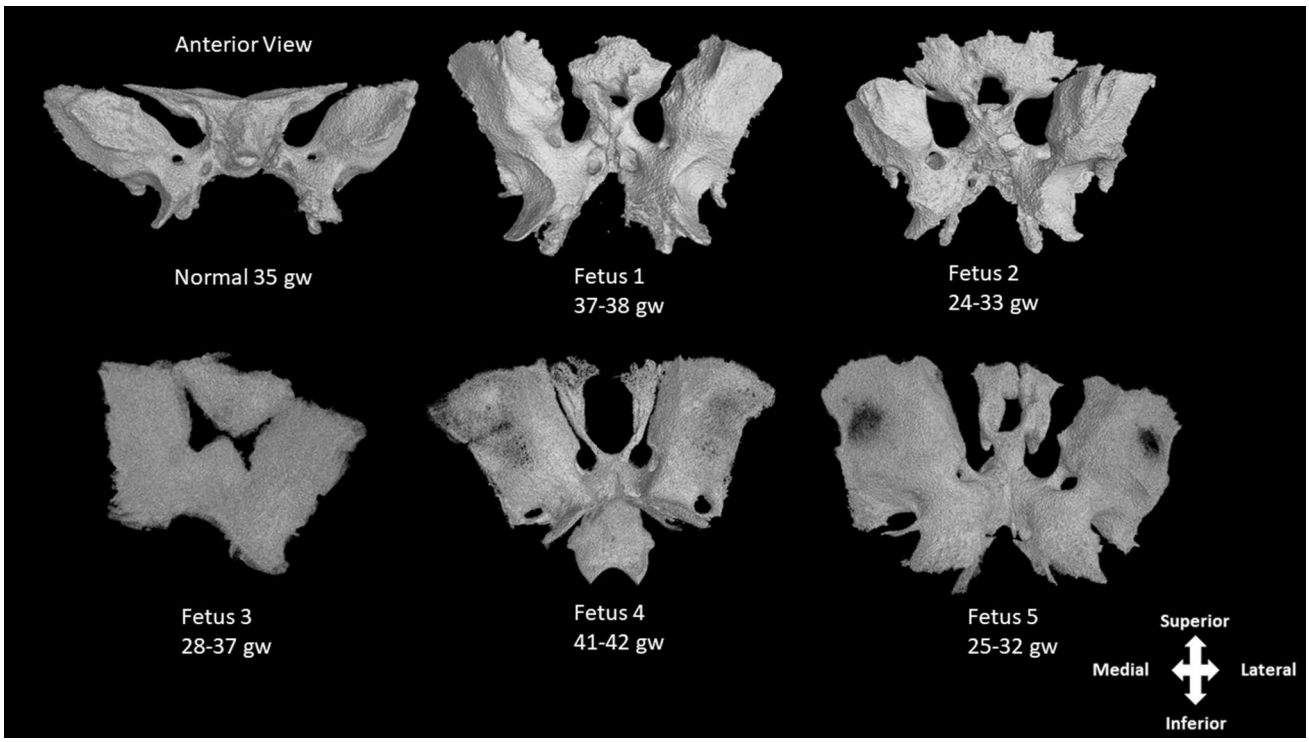

Fig. 5 Anterior view of the sphenoid bone of a normal fetus and the highly variable morphology of the sphenoid bones of the five cyclopean fetuses

However, a gap in the anterior part of the fused frontal bones for the ethmoid was discernible in fetuses 1 and 5 (Fig. 4). In relation to the nasal cavity, the lacrimal, the nasal, the inferior concha and the vomer bones were absent from all fetuses (Table 1).

In general, while the morphology of the maxilla was identifiable, there were significant variations in the dental configuration as a result of an abnormally formed premaxillary region. The premaxilla in early development is an independent bone. In the adult, its presence is indicated by the incisor teeth-bearing region of the hard palate (Table 1; Fig. 6). In fetuses 1 and 2, a central conoid incisor was observed with an absence of lateral incisors. However, canines were present as well as a full set of deciduous molars commensurate with the relevant stage of dental development. In contrast, in fetuses 3–5, both canines were present, while the central and lateral incisors were absent. Furthermore, the anterior nasal spine and frontal processes of the maxilla were abnormally developed across all individuals. Osteogenesis in the anterior region of the maxilla in fetus 2 was poor (Fig. 7).

The horizontal plates of the palatine bones were present and were in horizontal alignment. The greater and lesser palatine foramina were evident in fetuses 4 and 5 (Table 1). The perpendicular plates of the palatine bone fused centrally to form a midline vertical bony mass in all fetuses (Fig. 8). The mandible was generally not as adversely affected in terms of its morphology (Table 1), but significant crowding of the anterior dentition was evident.

Discussion

The analysis of the individual bones and the composite of the craniofacial skeleton in the cyclopean fetuses was markedly enabled by the three-dimensional reconstruction permitted by micro-CT scanning. This provided insight into both abnormal and normal development and growth of the region.

The most conspicuous osteological anomalies in the five fetuses with cyclopia were the single median orbit and the absence of the nasal cavity. The absence or malformation of the premaxilla, presphenoid, vomer, ethmoid, nasal bones, inferior conchae, lacrimal as well as the frontal bone concurs with descriptions in the literature [12, 18, 25]. The disturbance in the development of the latter structures resulted in the obliteration of the median wall of the orbital cavity and the entire nasal cavity. At the other end of the spectrum, the least affected bones were the temporal, occipital, parietal, zygomatic bones and the mandible. Thus, the midfacial skeleton of the fetuses were the elements most affected. The dysmorphogenesis appears to be related to the underlying

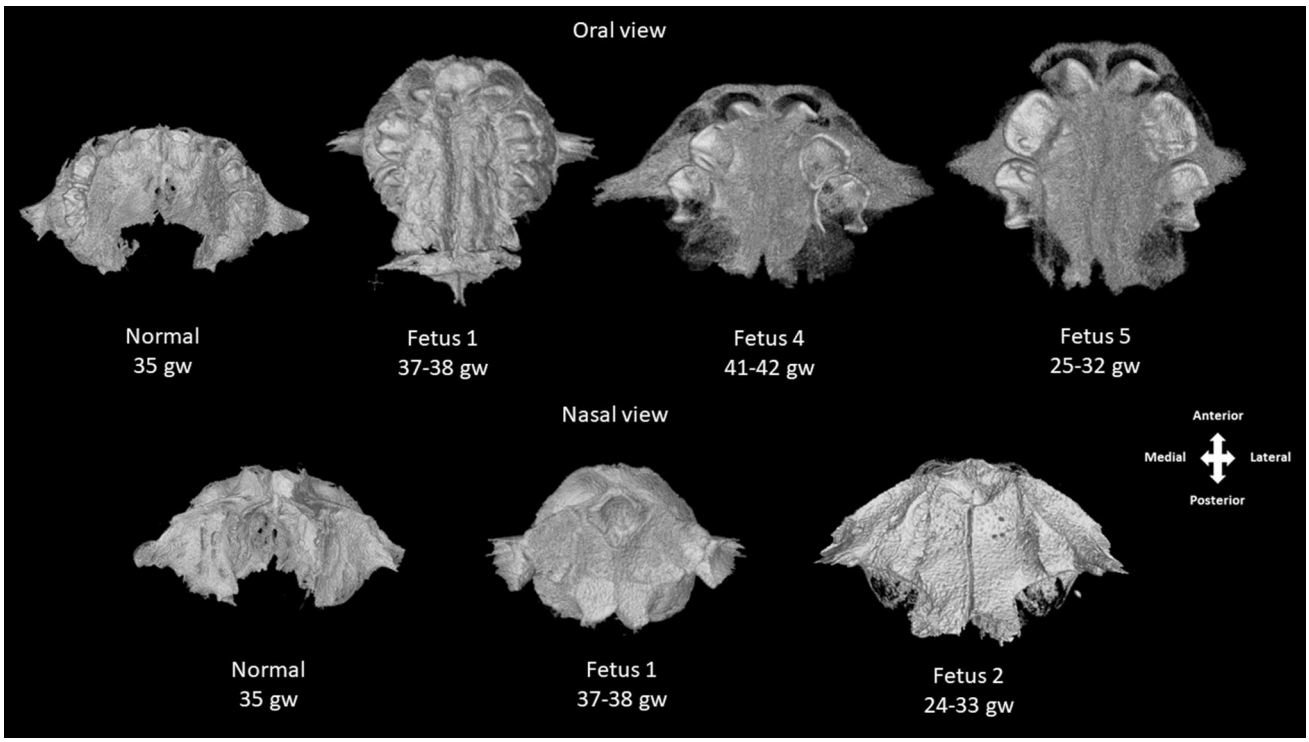

Fig. 6 An oral view of the maxillary bone of a normal fetus and three cyclopean fetuses illustrates the variation in the central incisor teeth. The nasal view of the normal and cyclopean fetuses indicating the variability of the nasal floor morphology

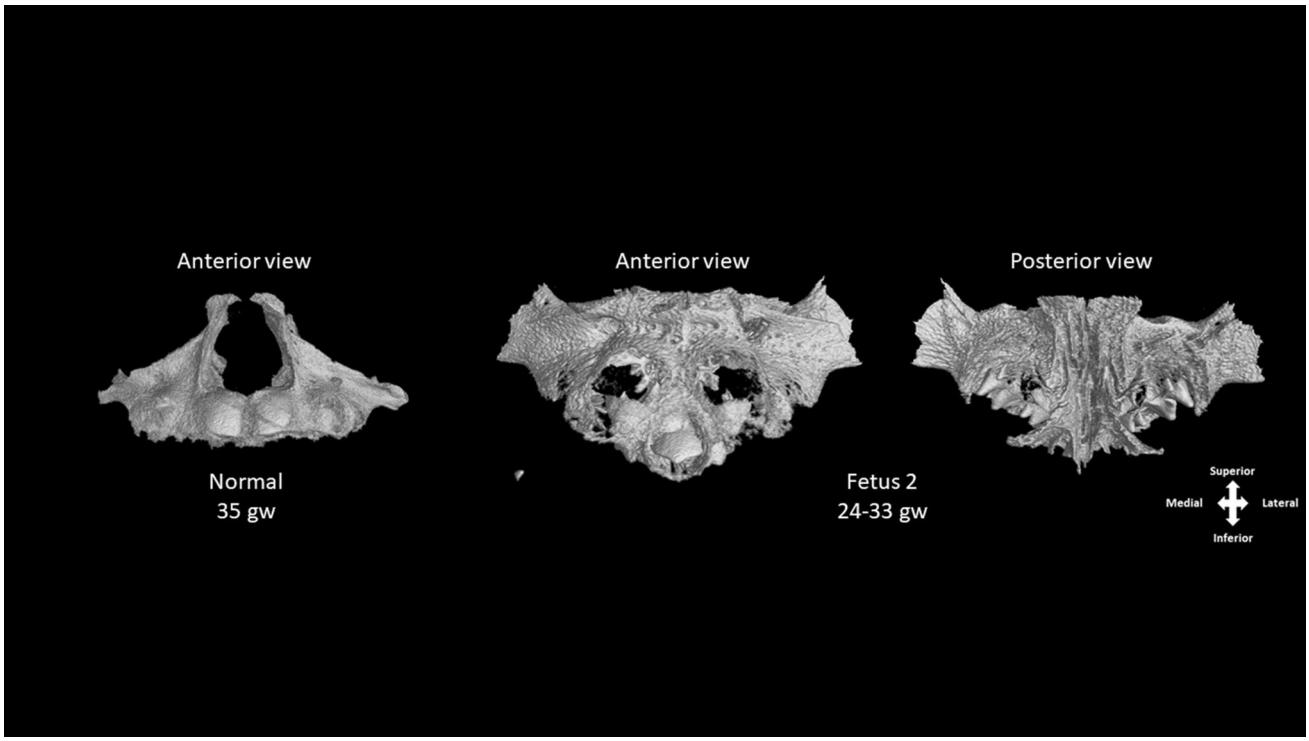

Fig. 7 The normal fetal maxilla (anterior view) and the maxilla of cyclopean specimen 2 anterior and posterior view) demonstrating the maldevelopment of the palatine bone in the cyclopean fetus

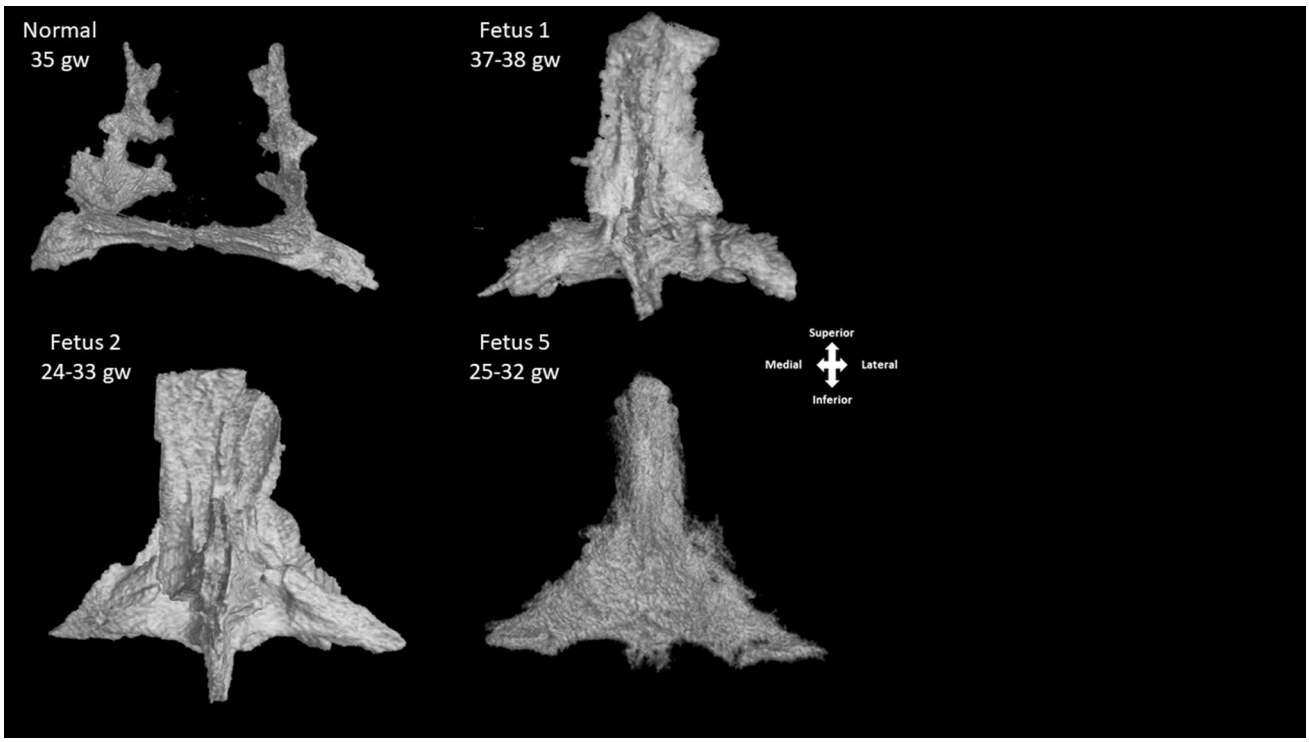

Fig. 8 Comparison of an anterior view of the perpendicular plates of the palatine bone in a normal fetus and three cyclopean fetuses. Note the fusion of these plates in the cyclopean fetuses centrally, to form a midline vertical bony mass

apparent holoprosencephaly, which causes maldevelopment of the frontonasal process and its derivatives [24].

The basicranium is integrated into the face by means of the perpendicular laminae and lateral masses of the ethmoid. Ethmoidal development appears to be critical for the development of the facial skeleton. The perpendicular plate of the ethmoid has been described as a midline strut which provides structural integrity to the developing facial bones [14]. This hypothesis is borne out in the many studies undertaken in cyclopean fetuses where the ethmoid is absent and the two sides of the midfacial region remain conjoined [12, 14, 18, 24, 25]. The ethmoid complex develops from the frontonasal process [26] and is heavily populated with neural crest cells. The ethmoid bone normally results from centres in the mesethmoid cartilage and nasal capsule cartilages [26]. Normally, the nasal cartilages span the gap between the basicranium and the viscerocranium, and separate bones within the nasal region [12]. As a result of the absence or malformation of ethmoid cartilage derivatives (nasal septal cartilage, cribriform plate, perpendicular plate, superior and middle conchae) in cyclopia, the malposition and fusion of midline facial bones occur [12]. Failure of development of the ethmoid complex, which normally specifies the two sides of the face, results in the dysmorphogenesis of the middle and "upper" face with no characterization of the median cranial plane [1]. This plane may be under the control of a variety of factors and genes such as SHH [8].

As portions of the craniofacial skeleton derived from the median nasal process, which originates from the frontonasal process, are often missing in cyclopia [12], it is not surprising that the premaxilla, also a derivative of the median nasal process, was abnormally developed in all cyclopean fetuses in this study. This was accompanied by abnormalities in the anterior dentition. The underdevelopment of the premaxilla coincided with either the presence or absence of the central incisor teeth [7, 10, 12]. As the development of the premaxillary bone is dependent on the presence of, and is secondary to the fusion of, the medial nasal mesenchyme [3, 12], the absence of the upper central incisors is not unexpected. However, the origin of the central conoid tooth is of interest and is somewhat controversial. Sedano and Gorlin [23] postulated that this tooth was due to the fusion of the two lateral incisors. However, Gardner and Lim [10] propose that this tooth is in fact a mesiodens (a supernumerary tooth) rather than the fusion of two teeth.

The most affected region of the basicranium in the cyclopean fetuses of this study was the centrally located presphenoid bone and its associated components. The malformation of the presphenoid appeared to have a cascading effect on the morphology and orientation of the greater and lesser wings. Abnormalities of the presphenoid bone may result from an imbalance in the neural crest cells forming the presphenoid cartilages [2, 12, 21]. Conventionally, the fused presphenoid cartilages are said to contribute to the formation of the mesethmoid cartilage [26]. Most anteriorly, the mesethmoid cartilage becomes a vertical plate within the nasal septum [26]. Therefore, it appears that the dysmorphogenesis of the presphenoid and mesethmoid cartilages affects the incidence and integrity of the nasal septum, which in turn affects the development of the midline structures of the viscerocranium [5, 12, 18, 21].

During normal development, the evagination of the paired telencephalic vesicles and paired olfactory regions from the prosencephalon is associated with the development of the prosencephalic centre at the rostral tip of the notochord. The prosencephalic centre and the rhombencephalic centre are together responsible for the normal development of the viscerocranium [26]. These two centres are regulated by SHH [26]. The prechordal plate is said to suppress the median part of the optic primordium so that bilateral primordia normally develop [19, 26, 28].

Mutation of genes (e.g. SHH) involved in cellular communication between the organizing centres and neural crest cells that populate the frontonasal process and its derivatives affects neural crest cell migration, condensation and chondrogenesis. Thus, a disruption of forebrain development not only produces holoprosencephaly, but also decreases the production of SHH [25]. Reduced expression of SHH in mutant mice has been correlated with varying degrees of holoprosencephaly and cyclopia [11]. Experimental evidence supports the hypothesis that perturbations in cholesterol biosynthesis, which result from either a "defect in SHH cholesterol autoprocessing", or an inability for the "target tissue to sense or transduce the SHH signal", cause holoprosencephaly [4, 8, 9].

Barteczko and Jacob [2] showed that the rostral tip of the notochord in human embryos is situated at the future region of the basioccipital and basisphenoidal bones. The spheno-occipital junction demarcates the prechordal from the chordal region [17]. In the five fetuses with cyclopia analysed in this study, the most affected craniofacial bones were located anterior to the spheno-occipital junction and in the midline region of the skull. These findings concur with the findings made by Barteckzo and Jacob [2] and support the hypothesis that cyclopia results from disturbances of the rostral region of the notochord.

Limitations

Developmental disturbances are helpful indicators in understanding normal development and growth. However, the limited numbers of abnormal fetuses in this study preclude the sectioning or dissection of these fetuses. This is restrictive, in that soft tissue associations could provide further insight into morphological changes which may occur over time.

Perspectives

The importance of the presphenoid and the ethmoid bone as midline struts in the development and growth of the craniofacial complex as well as the separation of the orbits is well illustrated by the analysis of cyclopean fetuses and provides an understanding of normal craniofacial growth.

Conclusion

The micro-CT assessment provided information not only on the incidence of malformed structures but also on the degree of malformation and the resultant effect of these on neighbouring structural relationships. Thus, non-separation of the underlying prosencephalon resulted in dysmorphologies of median plane structures of the cranium. The bones forming the nasal cavity and the presphenoid bone were most affected suggesting that the defective development of the presphenoid cartilage was largely responsible for the maldevelopment of the cartilages giving rise to components of the nasal cavity. The role of the presphenoid and ethmoid bone as important midline struts in the normal development of the human face is emphasized by the non-laterality seen in cyclopean fetuses.

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Author contributions BK: project development, data analysis and manuscript writing. KM: data collection and data analysis. EFH: project development, data analysis and manuscript editing.

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